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Review Article

Capsules, Fecal Microbiota Transplantation, or Freeze-Drying?

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Abstract

A comprehensive review of the world literature was conducted to determine which of the three currently available methodologies is the best. All concepts were analyzed when transplanting the gut microbiota from a healthy individual to a patient with pseudomembranous enterocolitis due to *Clostridium difficile*, irritable bowel syndrome, inflammatory bowel disease, and other disorders.

Keywords: *Capsules (Caps); FMT (Fecal Microbiota Transplantation); Lyophilization (Lio)*

Introduction

The concepts we considered were weight, patient acceptance, technical difficulty, cost, probable complications, improvement, and the need for future repeat procedures. Although these are crucial factors in the procedure, we did not delve into the donors or the studies performed on them, as we believe this can be omitted, given that the literature requires a significant number of laboratory tests and screenings, all of which are quite similar. Among the procedures performed, we analyzed endoscopic processes, administration via nasogastric tube, nasojejunal tube, and duodenal infusion; application via retention enema; and capsules, whether containing frozen, lyophilized, or fresh stool. We began the evaluation with transplantation via upper gastrointestinal endoscopy, colonoscopy, or a combination thereof, followed by the use of nasogastric, duodenal, or jejunal tubes. Retention enemas and capsules, frozen or not, prepared by the team involved in the procedure or by a specialized laboratory, including whether they are lyophilized or not. Administration as frozen stool is also specified, emphasizing that there are different ways to use it, which makes them non-uniform. However, we strive to abbreviate the concepts to provide a better frame of reference. Finally, lyophilization is defined, and its direct effect on the results is considered.

General concepts

Fecal microbiota transplantation (FMT) can be administered via the upper gastrointestinal tract (endoscopically or using a nasogastric, nasoduodenal, or nasojejunal tube); via the lower gastrointestinal tract (retention enema, colonoscopy); or via capsules (Caps), frozen or not, containing lyophilized or non-lyophilized fecal matter [1].

Endoscopic procedures

These can be performed via panendoscopy, colonoscopy [2], or a combination of both. We prefer panendoscopy that reaches the jejunum. The microbiota is deposited there, as three strictures prevent reflux: the ligament of Treitz, the pylorus, and the lower esophageal

sphincter [3]. Colonoscopy is the most widely used procedure, but capsule administration is gradually becoming more common due to its ease of administration [4].

Administration via tubes

The nasogastric, nasoduodenal, and nasojejunal routes have been recommended, with the latter being preferred for the same reasons explained above. Instillation of stool through the nasogastric tube could potentially cause aspiration of fecal material, and therefore it is reasonable to consider the need for a PA chest X-ray to ensure that the catheter is in place [5].

Retention enema

A procedure used in the United States in 1958 by a surgeon in Denver, Colorado, for pseudomembranous colitis, with very good results. This procedure is currently recommended as a follow-up treatment after colonoscopy, given the existence of conditions that may contraindicate the enema [6].

Oral capsules

Developed in July 2010 to treat patients who could not tolerate a jejunal catheter due to esophageal varices or the inability to retain fecal enemas due to anal incontinence. They have even been used to eradicate carbapenemase-producing enterobacteria colonization, although they have been most frequently used for the eradication of *C. difficile*, using 100 grams of freshly collected stool; 15 capsules daily for two days on an empty stomach [7].

Preparation of lyophilized fecal microbiota capsules

The lyophilized product was prepared by dilution and homogenization, filtration to remove fibrous material, concentration of the microbiota by centrifugation, and lyophilization of the concentrated pellet. Since microorganisms are key to therapeutic activity, the procedure was designed to separate the microorganisms from non-viable feces using an efficient, well-controlled, and reproducible process, concentrating them in a way that preserves their viability and allows for administration, even in single doses [8].

Fresh or frozen capsules

These variants, with numerous successes, are part of the current therapeutic arsenal. Therefore, they can be used and offered as an option, if available, in cases of intolerance to the jejunal catheter (esophageal varices) or if the patient cannot retain the enema due to anal incontinence. Likewise, they should be avoided in frail elderly patients who have difficulty swallowing so many capsules. On the other hand, frozen whole feces are thawed and processed, which is equivalent to the classic preparation, and the number of live cells decreases from 75% to 15%. A cryoprotectant must be included so that the bacterial community is not negatively impacted, as well as its viability [9].

Lyophilization

Also known as freeze-drying or cryodesiccation, this is a low-temperature dehydration process that freezes the product, lowers the pressure, and removes the ice through sublimation, without passing through the liquid state. This process can resolve *Clostridium difficile* diarrhea with the administration of 4 to 5 lyophilized capsules [8]. However, the question of its long-term efficacy for the microbiome remains open. Reygner J and his group state that "both lyophilization and freezing allow for the preservation of viability," confirming this through the concentration of fatty acids over time [10].

Lyophilized and non-lyophilized capsules are available, including frozen and unfrozen versions. Their use should be avoided if the patient has a history of anaphylactic food allergies. Meanwhile, Sedeek SA and his team point out that: "Freeze-dried fecal microbiota is an alternative to frozen products," and furthermore, when applied, it shows higher counts of viable bacterial cells and anaerobes, compared to fresh stool. There is no effect on bile acids or 16S rRNA [11].

Freeze-dried capsules versus frozen enema

A meta-analysis found that administering 100 to 200 grams of frozen stool via enema to patients with recurrent *C. difficile* infection resulted in 88% improvement. The capsules improved the condition in 84% of patients, which is considered equivalent efficacy [12].

Freeze-dried capsules versus frozen microbiota

Frozen microbiota therapy (FMT) is prepared by freezing a filtered stool solution and then lyophilizing it by sublimation into a powder, which is easier to encapsulate. No statistically significant differences were reported between the two procedures when applied to recurrent *C. difficile* infection (20,623 patients) and irritable bowel syndrome [13].

Freeze-dried capsules

The multiple lines of evidence that FMT can be administered via oral, frozen, and freeze-dried capsules represent significant advances. These capsules have been administered in quantities of 20 to 40, each containing 60 mg of feces, prepared with 40 grams of feces. The authors suggest administering it in frozen capsules, as has been done by several other authors, and they determine that the effect is similar with greater stability. Regarding freeze-dried capsules, they have treated 20 patients with good results [14].

Capsules vs. colonoscopy

Positive results have been observed in both procedures in up to 96.2% of cases, with no inferiority in either regimen. Even with strict environmental control, administering the capsules within 6 hours of the start of preparation yields very similar results [15].

Viability of freeze-dried transplants

Methods have been sought to prevent the reduction of the diversity and potency of microorganisms, since it is known that when prepared in the environment, 50% of the bacteria are not viable [16]. Given that there are currently more than 200 protocols worldwide aiming to minimize this issue, we considered it important to examine this undeniable matter to explore how it can be addressed. This could involve using sequential labeling with D-amino acid-based metabolic probes (STAMP), homogenizing the ambient air, or freezing and thawing. It is also worth noting that transplants prepared in maltodextrin-trehalose solutions, stored in a standard freezer at -80°C and immediately thawed at 37°C, retained the best reactivation potential [17].

Frozen microbiota

This method, like all others, has not demonstrated superiority, as all pathways used in fecal microbiota transplantation (FMT) yield similar and positive results. It involves three aspects: donor selection, preparation of the substance, and delivery. Frozen donor stool samples contained viable bacteria for up to two years, similar to those of freshly obtained samples, leading to the conclusion that stool storage does not compromise success, despite taxonomic changes [18].

Fresh stool

The use of fresh stool for bacterial transplantation was the most effective treatment for recurrent *Clostridium difficile* infection compared to antibiotic therapy or placebo. When compared to stool frozen at -30°C without processing or additives, the results are equivalent. It is worth reiterating that the results of frozen stool without cryoprotectants have an impact on cultures and the diversity of the bacterial community [19].

Frozen stool vs. fresh stool

Frozen stool samples are as effective as fresh stool samples. The effectiveness of both procedures is particularly evident in recurrent *C. difficile* infection. An oxygen-free atmosphere is not necessary, and removing the air above the collected samples is sufficient to preserve

the viability of the frozen capsules. In 219 patients, clinical resolution was 83.4% with frozen stool samples and 85.1% with fresh stool samples. This indicates that both processes produce very similar cure rates [20].

Standardization of frozen stool samples

At the University of Minnesota, they have frozen stool samples that remain effective. Therefore, Takahashi M and his team [21] emphasize the importance of short-term freezing, the period during which enteric colonization capacity is maintained; reiterating that long-term storage of stool at -20°C can cause instability.

Side effects

We detected few complications, and aside from aspiration, they are very similar, infrequent, and, in most cases, reversible.

Costs

Endoscopic procedures are slightly more expensive; however, the ability to visualize the area where stool is deposited and to diagnose clinically undetected processes justifies the increased cost. We believe that other procedures can be performed once the endoscopic procedure has been used. The cost of gut microbiota transplantation is expected to be lower compared to the cost of repeated antibiotic therapy, hospitalization, and lost work productivity due to persistent diarrhea [22].

Conclusion

The use of any procedure is safe in susceptible conditions, such as recurrent *C. difficile* infection, and its application is expected to increase in other conditions in the near future.

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